

Appl. No. 09/546,857

Amdt. dated May 21, 2004

Reply to Final Office Action of January 21, 2004

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

Claims 1-19 (canceled)

Claim 20 (currently amended): A VEGF variant polypeptide comprising amino acid substitutions at residues corresponding to amino acid residues 63, 65, and 66 of human VEGF wherein the amino acid substitutions are D63S, G65M, and L66R.

Claim 21 (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim 20.

Claim 22 (previously presented): A vector comprising the nucleic acid of claim 21.

Claim 23 (currently amended): A VEGF variant polypeptide comprising one or more amino acid substitutions at residues corresponding to amino acid residues 63 to 66 of native human VEGF and one or more amino acid substitutions at residues corresponding to amino acid residues 17 to 25-18, 21, 22, or 25 of native human VEGF, wherein the VEGF variant polypeptide has selective binding affinity for KDR receptor as compared to native human VEGF.

Claim 24 (canceled)

Claim 25 (previously presented): The VEGF variant of claim 23, wherein the amino acid substitution(s) comprises D63S, G65M, or L66R.

Claim 26 (currently amended): The VEGF variant of claim ~~25~~23, wherein the amino acid substitution(s) ~~further~~ comprises M18E, Y21L, Q22R, or Y25S.

Claim 27 (currently amended): The VEGF variant of claim ~~25~~23, wherein the amino acid substitutions ~~further~~ comprise M18E, Y21L, Q22R, and Y25S.

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Claim 28 (currently amended): The VEGF variant of claim 23 wherein the amino acid substitutions comprise[s] D63S, G65M, and L66R

Claim 29 (currently amended): The VEGF variant of claim 28, wherein the amino acid substitution(s) ~~further~~ comprise M18E, Y21L, Q22R, or Y25S.

Claim 30 (currently amended): The VEGF variant of claim 28, wherein the amino acid substitutions ~~further~~ comprise M18E, Y21L, Q22R, and Y25S.

Claim 31 (previously presented): A VEGF variant of claim 23, comprising one of the following combinations of amino acid substitutions:

- (a) M18E, D63S, G65M, and L66R;
- (b) Y21L, D63S, G65M, and L66R;
- (c) Q22R, D63S, G65M, and L66R;
- (d) Y25S, D63S, G65M, and L66R;
- (e) M18E, Y21L, D63S, G65M, and L66R;
- (f) M18E, Q22R, D63S, G65M, and L66R;
- (g) M18E, Y25S, D63S, G65M, and L66R;
- (h) Y21L, Q22R, D63S, G65M, and L66R;
- (i) Y21L, Y25S, D63S, G65M, and L66R;
- (j) Q22R, Y25S, D63S, G65M, and L66R;
- (k) M18E, Y21L, Q22R, D63S, G65M, and L66R;
- (l) M18E, Q22R, Y25S, D63S, G65M, and L66R;
- (m) Y21L, Q22R, Y25S, D63S, G65M, and L66R;
- (n) M18E, Y21L, Q22R, Y25S, and D63S;
- (o) M18E, Y21L, Q22R, Y25S, and G65M;
- (p) M18E, Y21L, Q22R, Y25S, and L66R;
- (q) M18E, Y21L, Q22R, Y25S, D63S, and G65M;
- (r) M18E, Y21L, Q22R, Y25S, D63S, and L66R;

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- (s) M18E, Y21L, Q22R, Y25S, G65M, and L66R; or
- (t) M18E, Y21L, Q22R, Y25S, D63S, G65M, and L66R.

Claim 32 (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim 23.

Claim 33 (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim 27.

Claim 34 (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim 29.

Claim 35 (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim 31.

Claim 36 (previously presented): A vector comprising the nucleic acid of claim 32.

Claim 37 (previously presented): A host cell comprising the vector of claim 36.

Claim 38 (previously presented): A composition comprising the VEGF variant of claim 23 and a carrier.

Claim 39 (previously presented): The composition of claim 38, wherein the carrier is a pharmaceutically acceptable carrier.

Claim 40 (previously presented): An assay for detecting KDR receptor, comprising contacting an isolated cell or tissue with a VEGF variant of claim 23 and assaying for binding of the VEGF variant to the cell or tissue.

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Claim 41 (previously presented): A method for stimulating phosphorylation of a KDR receptor, comprising contacting a cell with a VEGF variant of claim 23 in amount effective to stimulate phosphorylation of the KDR receptor.

Claim 42 (previously presented): A method for stimulating MAP kinase activation, comprising contacting a cell with a VEGF variant of claim 23 in amount effective to stimulate phosphorylation of MAP kinase.

Claim 43 (previously presented): A method for stimulating PLC-gamma activation, comprising contacting a cell with a VEGF variant of claim 23 in amount effective to stimulate phosphorylation of PLC-gamma.

Claim 44 (previously presented) A method for stimulating PI 3'-kinase activation, comprising contacting a cell with a VEGF variant of claim 23 in amount effective to stimulate phosphorylation of PI 3'-kinase.

Claim 45 (previously presented): A method for stimulating vasculogenesis or angiogenesis, comprising contacting a endothelial cells expressing KDR receptor with an effective amount of a VEGF variant of claim 23.

Claim 46 (previously presented): A method for promoting the migration of endothelial cells, comprising contacting endothelial cells expressing KDR receptor with an effective amount of a VEGF variant of claim 23.

Claim 47 (currently amended): A VEGF variant polypeptide comprising ~~two~~one or more amino acid substitutions at residues corresponding to amino acid residues 17 to 25 of native human VEGF, wherein the VEGF variant polypeptide has selective binding affinity for KDR receptor as compared to native human VEGF.

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Claim 48 (currently amended): The VEGF variant of claim 47, wherein the amino acid substitution(s) substitutions comprise ~~two or~~ one or more amino acid substitutions at the residues corresponding to ~~at~~ amino acid residues 18, 21, 22, or 25 of native human VEGF.

Claim 49 (previously presented): The VEGF variant of claim 47, wherein the amino acid substitution(s) comprise M18E, Y21L, Q22R, or Y25S.

Claim 50 (previously presented): The VEGF variant of claim 47, wherein the amino acid substitutions comprise M18E, Y21L, Q22R, and Y25S.

Claim 51 (previously presented): The VEGF variant of claim 47, wherein the amino acid substitutions comprise F17I, M18E, Y21F, Q22K, and Y25S.

Claim 52 (previously presented): The VEGF variant of claim 47, wherein the amino acid substitutions comprise F17I, M18E, Y21F, Q22E, and Y25I.

Claim 53 (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim 47.

Claim 54 (previously presented): An isolated nucleic acid sequence encoding the VEGF variant of claim 50.

Claim 55 (new): The VEGF variant of claim 23, wherein the amino acid substitutions further comprise a substitution at the residue corresponding to amino acid residue 17 of native human VEGF.

Claim 56 (new): A VEGF variant polypeptide, comprising:

(a) one or more amino acid substitutions at residues corresponding to amino acid residues 17-25 of native human VEGF, and

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(b) one or more amino acid substitutions at residues corresponding to amino acid residues 63-66 of native human VEGF;
wherein amino acid residue 60 is cysteine.